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Patent Application
Docket No. UF-221C1XC1
Serial No. 09/662,254

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Examiner	:	Anne Marie S. Beckerleg, Ph.D.
Art Unit	:	1632
Applicants	:	Richard W. Moyer, Yi Li, Allison L. Bawden
Serial No.	:	09/662,254
Filed	:	September 14, 2000
Confirm. No.	:	2442
For	:	Materials and Methods for Delivery and Expression of Heterologous DNA in Vertebrate Cells

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF RICHARD W. MOYER, Ph.D., UNDER 37 C.F.R. § 1.132

Sir:

I, Richard W. Moyer, Ph.D., of the University of Florida, hereby declare:

THAT, I am an inventor on the above-referenced patent application;

THAT, I have received the following degrees:

B.S. in Agricultural and Biological Chemistry with Minor in Chemistry; Pennsylvania State University; 1962

Ph.D. in Biochemistry with Minor in Organic Chemistry; University of California at Los Angeles; 1967;

THAT, I have been employed professionally as follows:

1987-present	Professor and Chairman, Department of Immunology and Medical Microbiology, College of Medicine, University of Florida

1981-1987	Professor, Department of Microbiology, Vanderbilt University School of Medicine
1976-1981	Associate Professor, Department of Microbiology, Vanderbilt University School of Medicine
1970-1976	Assistant Professor of Biochemistry, Columbia University
1967-1969	Postdoctoral Fellow, Massachusetts Institute of Technology
6/1965-9/1965	Summer Trainee in Physiology, Woods Hole Marine Biology Laboratory
1963-1967	Research Assistant, Biochemistry, University of California at Los Angeles
1962-1963	Research Assistant, Biochemistry, University of Minnesota

THAT, I have published extensively in my field and some of the publications are as follows:

1. Bawden, A.L., K.J. Glassberg, J. Diggans, R. Shaw, W. Farmerie, R.W. Moyer (2000) "Complete genomic sequence of the *Amsacta moorei* entomopoxvirus: Analysis and comparison with other poxviruses" *Virology* 274(1):120-139;

2. Moon, K.B., P.C. Turner, R.W. Moyer (1999) "SPI-1-dependent host range of rabbitpox virus and complex formation with cathepsin G is associated with serpin motifs" *Journal of Virology* 73(11):8999-9010;

3. Turner, P.C., M.S. Sancho, S.R. Thoennes, A. Caputo, R.C. Bleackley, R.W. Moyer (1999) "Myxoma virus Serp2 is a weak inhibitor of granzyme B and interleukin-1beta-converting enzyme *in vitro* and unlike CrmA cannot block apoptosis in cowpox virus-infected cells" *Journal of Virology* 73(8):6394-6404;

4. Li, Y., S. Yuan, R.W. Moyer (1998) "The non-permissive infection of insect (gypsy moth) LD-652 cells by Vaccinia virus" *Virology* 248(1):74-82;

5. Li, Y. R.L. Hall, S.L. Yuan, R.W. Moyer (1998) "High-level expression of *Amsacta moorei* entomopoxvirus spheroidin depends on sequences within the gene" *J Gen Virol* 79(Pt 3):613-622;

6. Li, Y. R.L. Hall, R.W. Moyer (1997) "Transient, nonlethal expression of genes in vertebrate cells by recombinant entomopoxviruses" *Journal of Virology* 71(12):9557-9562;

7. Peterson, B.O., R.L. Hall, R.W. Moyer, S. Shuman (1997) "Characterization of a DNA topoisomerase encoded by *Amsacta moorei* entomopoxvirus" *Virology* 230(2):197-206;

8. Graham, K.A., A.S. Lalani, J.L. Macen, T.L. Ness, M. Barry, L.Y. Liu, A. Lucas, I. Clark-Lewis, R.W. Moyer, G. McFadden (1997) "The T1/35kDa family of poxvirus-secreted proteins bind chemokines and modulate leukocyte influx into virus-infected tissues" *Virology* 229(1):12-24;

9. Winter, J., R.L. Hall, R.W. Moyer (1995) "The effect of inhibitors on the growth of the entomopoxvirus from *Amsacta moorei* in Lymantria dispar (gypsy moth) cells" *Virology* 211(2):462-473;

10. Turner, P.C., P.Y. Musy, R.W. Moyer (1994) *Viroceptors, Virokines, and Related Immune Modulators Encoded by DNA Viruses* (McFadden, G., ed.), R.G. Landes, Galveston, TX;

11. VanderLeek, M.L., J.A. Feller, G. Sorensen, W. Isaacson, C.L. Adams, D.J. Borde, N. Pfeiffer, T. Tran, R.W. Moyer, E.P.J. Gibbs (1994) "Evaluation of swinepox virus as a vaccine vector in pigs using an Aujeszky's disease (pseudorabies) virus gene insert coding for glycoproteins gp50 and gp63" *The Veterinary Record* 134:13-18;

12. Moyer, R.W. (1994) *Encyclopedia of Virology* (Webster, R.G., A. Granoff, eds.), Academic Press Ltd., London: pp. 392-397;

13. Ali, A.N., P.C. Turner, M.A. Brooks, R.W. Moyer (1994) "The SPI-1 gene of rabbitpox virus determines host range and is required for hemorrhagic pock formation" *Virology* 202:306-314;

14. Martinez Pomares, L., R.J. Stern, R.W. Moyer (1993) "The ps/hr gene (B5R open reading frame homolog) of rabbitpox virus controls pock color, is a component of extracellular enveloped virus, and is secreted into the medium" *Journal of Virology* 67:5450-5462;

15. Hall, R.L., R.W. Moyer (1993) "Identification of an *Amsacta* spheroidin-like protein within the occlusion bodies of *Choristoneura* entomopoxviruses" *Virology* 192:179-187

16. Bloom, D.C., R. Stern, M. Duke, D. Smith, R.W. Moyer (1993) "A revised *Hind*III map and sequence analysis of a large 'left-hand' non-essential region of the rabbit poxvirus genome" *Virus Res* 28:125-140;

17. Thompson, J.P., P.C. Turner, A.N. Ali, B.C. Crenshaw, R.W. Moyer (1993) "The effects of serpin gene mutations on the distinctive pathobiology of cowpox and rabbitpox virus following intranasal inoculation of Balb/c mice" *Virology* 197:328-338;

18. Massung, R.F., V. Jayarama, R.W. Moyer (1993) "DNA sequence analysis of conserved and unique regions of swinepox virus: Identification of genetic elements supporting phenotypic observations including a novel G protein-coupled receptor homologue" *Virology* 197:511-528;

19. Turner, P.C., R.W. Moyer (1992) "An orthopoxvirus serpinlike gene controls the ability of infected cells to fuse" *Journal of Virology* 66:2076-2085;

20. Turner, P.C., R.W. Moyer (1992) "A PCR-based method for manipulation of the vaccinia virus genome that eliminates the need for cloning" *Biotechniques* 13:764-771;

21. Massung, R.F., G. McFadden, R.W. Moyer (1992) "Nucleotide sequence of a unique near-terminal region of the tumorigenic poxvirus, Shope fibroma virus" *J Gen Virology* 73:2903-2911;

22. Gruidl, M.E., R.L. Hall, R.W. Moyer (1992) "Mapping and molecular characterization of a functional thymidine kinase from *Amsacta moorei* entomopoxvirus" *Virology* 186:507-516;

23. Hall, R.L., R.W. Moyer (1991) "Identification, cloning, and sequencing of a fragment of *Amsacta moorei* entomopoxvirus DNA containing the spheroidin gene and three vaccinia related ORFs" *Journal of Virology* 65:6516-6527;

24. Feller, Moyer *et al.* (1991) "Isolation of molecular characterization of the swinepox virus thymidine kinase gene" *Virology* 183:578-585;

25. Brown, C.K., D.C. Bloom, R.W. Moyer (1991) "The nature of naturally occurring mutations in the hemagglutinin gene of vaccinia virus and the sequence of immediately adjacent genes" *Virus Genes* 5:235-242;

26. Brown, C.K., P.C. Turner, R.W. Moyer (1991) "Molecular characterization of the vaccinia hemagglutinin gene" *Journal of Virology* 65:3598-3606;

27. Bloom, D.C., K.M. Edwards, C. Hager, R.W. Moyer (1991) "Identification and characterization of two non-essential regions of the rabbitpox virus genome involved in virulence" *Journal of Virology* 65:1530-1542;

28. Massung, R.F., R.W. Moyer (1991) "The molecular biology of swinepox virus II. The infectious cycle" *Virology* 180:355-364;

29. Massung, R.F., R.W. Moyer (1991) "The molecular biology of swinepox virus I. Characterization of the viral DNA" *Virology* 180:347-354;

30. Turner, P.C., R.W. Moyer (1990) "The Molecular Pathogenesis of Poxviruses," in *Current Topics in Microbiology and Immunology*, P.C. Turner and R.W. Moyer, eds., Springer Verlag, New York, pp.125-153;

31. Turner, P.C., R.W. Moyer, eds. (1990) "The Molecular Pathogenesis of Poxviruses," in *The Poxviruses*, Springer Verlag, New York, pp.125-153;

32. Massung, R.F., R.W. Moyer (1989) "Orthopoxvirus gene expression in *Xenopus laevis* oocytes. I. A component of the virion is needed for late gene expression" *Journal of Virology* 64:2280-2289;

33. Turner, P.C., D.V. Young, J.B. Flanegan, R.W. Moyer (1989) "Interference with vaccinia virus growth caused by insertion of the coding sequences for poliovirus protease 2A" *Virology* 173:509-521;

34. Bloom, D.C., R. Massung, L. Savage, D.K. Morrison, R.W. Moyer (1989) "Recruitment to the cytoplasm of a cellular lamin-like protein from the nucleus during a poxvirus infection" *Virology* 169:115-126;

35. Edwards, K.M., T.C. Andrews, J. Van Savage, P. Palmer, R.W. Moyer (1988) "Poxvirus deletion mutants: Virulence and immunogenicity" *Microbial Pathogenesis* 4:325-333;

36. Moyer, R.W. (1987) "The role of the host cell nucleus in vaccinia virus morphogenesis" *Virus Research* 8:173-191;

37. Morrison, D.K., R.W. Moyer (1986) "Detection of a subunit of cellular PolII within highly purified preparations of RNA polymerase isolated from rabbit poxvirus virions" *Cell* 44:587-596;

38. Minnigan, H., R.W. Moyer (1985) "Intracellular location of rabbit poxvirus nucleic acid within infected cells as determined by *in situ* hybridization" *Journal of Virology* 55:634-643;

39. Brown, G.D., R.W. Moyer (1983) "The white pock mutants of rabbit poxvirus. V. *In vitro* translation of early host range mutant mRNA" *Virology* 126:381-390;

40. Moyer, R.W., R.L. Graves (1982) "The white pock mutants of rabbit poxvirus. IV. The late white pock (μ) host range (hr) mutants of rabbit poxvirus are blocked at the level of morphogenesis" *Virology* 119:332-346;

41. Moyer, R.W., R.L. Graves (1981) "The mechanism of cytoplasmic orthopoxvirus DNA replication" *Cell* 27:391-401;

42. Moyer, S.A., S.M. Horikami, R.W. Moyer (1981) "The effect of the host cell and heterologous viruses on VSV production," In *Replication of Negative Strand Viruses* (D.H.L. Bishop and R.W. Compans, eds.), Elsevier Press, New York, pp. 965-970;

43. Hamilton, D.H., R.W. Moyer, S.A. Moyer (1980) "Characterization of the non-permissive infection of rabbit cornea cells by vesicular stomatitis virus" *J Gen Virol* 49:273-287;

44. Moyer, R.W., R.L. Graves, C.T. Rothe (1980) "The white pock mutants of rabbit poxvirus. III. Terminal DNA sequence duplication and transposition in rabbit poxvirus" *Cell* 22:545-553;

45. Moyer, R.W., G.D. Brown, R.L. Graves (1980) "The white pock mutants of rabbit poxvirus. II. The early white pock (μ) host range (hr) mutants of rabbit poxvirus uncouple transcription and translation in non-permissive cells" *Virology* 106:234-249;

46. Moyer, R.W., C.T. Rothe (1980) "The white pock mutants of rabbit poxvirus. I. Spontaneous host range mutants contain deletions" *Virology* 102:119-132;

47. Herman, R.C., R.W. Moyer (1975) "*In vivo* repair of bacteriophage T5 DNA: An assay for viral growth control" *Virology* 66:393-407;

48. Moyer, R.W., A.S. Fu, J. Szabo (1972) "Regulation of bacteriophage development by Col I factors" *Journal of Virology* 9:804-812;

49. Moyer, R.W., R. Ramaley, P.D. Boyer (1967) "The formation and reactions of a nonphosphorylated high energy form of succinyl coenzyme A synthetase" *J Biol Chem* 242:4299; and

50. DeLuca, M., K.E. Ebner, D.E. Hultquist, G. Kreil, J.B. Peter, R.W. Moyer, P.D. Boyer (1963) "The isolation and identification of phosphohistidine from mitochondrial protein" *Biochem. Z.* 338:512;

THAT, I am an inventor on the following patents: U.S. Patent No. 5,935,777, "Entomopoxvirus Expression System," issued August 10, 1999; U.S. Patent No. 5,721,352, "Entomopoxvirus Expression System," issued February 24, 1998; U.S. Patent No. 5,651,972, "Use of Recombinant Swine Poxvirus as a Live Vaccine Vector," issued July 29, 1997; U.S. Patent No. 5,476,781, "Entomopoxvirus Spheroidin Gene Sequences," issued December 19, 1995; U.S. Patent 5,212,057, "Biological Systems for Constructing and Testing Viral Vaccines," issued May 18, 1993;

THAT, through my years of research, I have kept up to date on the technical literature and maintained contact with experts in the field by participating in professional meetings and seminars, and by direct personal contact. As a result, I am familiar with the general level of skill of those working in the fields of virology and molecular biology, and in particular the use of viral vectors in genetic engineering.

THAT, I have read and understood the specification and claims of the subject application and the Office Action dated September 4, 2001;

AND, being thus duly qualified, do further declare:

1. The entomopox virus vectors disclosed and claimed in this patent application have been found to be highly effective in delivering polynucleotides to vertebrate cells, resulting in the expression of genes encoded by the polynucleotides.

2. The patent Examiner states that at pages 13-14, bridging paragraph, of the patent application, it is indicated that entomopoxvirus cannot productively infect

mammalian cells and that gene expression is limited to early promoter activity. The patent Examiner also notes that the patent application indicates that late poxvirus promoters, such as AmEPV spheroidin or cowpox virus ATI, are inactive in mammalian cells infected with recombinant EPV. The patent Examiner then concludes "the skilled artisan would not predict that any and all promoter sequences could express a heterologous gene of interest when encoded by a recombinant entomopox virus."

3. The paragraph bridging pages 13-14 of the patent application refers to the general observation that when using entomopoxvirus vectors containing entomopox promoters, genes under the control of early entomopox promoters will be expressed in a vertebrate cell, but genes under the control of late entomopox promoters will not. The same paragraph of the patent application refers to two publications (Li *et al.* [1997] and Gauthier *et al.* [1995]), both of which describe experiments using entomopox virus vectors containing entomopox promoters. However, as explained below, the distinction between "early" promoters and "late" promoters only finds context with respect to poxvirus promoters (vertebrate and insect poxvirus promoters).

4. When insect poxviruses infect vertebrate cells, early and only early poxvirus promoters are active. This is likely because the early poxvirus transcription apparatus is packaged within the virion particle as part of creating virions from the previous infection. In vertebrate cells, following early promoter-driven expression, the infection then aborts and eventually the input virus particles disintegrate, after which the viral DNA is released into the vertebrate cell's cytoplasm; hence, the lack of late poxvirus promoter-driven expression in vertebrate cells. This observation is also made within the Li *et al.* publication (1997) at page 9557, second column, page 9560, second column, and page 9561, second column, which is cited by the patent Examiner. As indicated within the Li

et al. publication, at page 9561, second paragraph, and at page 71, lines 3-7, of the patent application, an exception to this phenomenon is when cells infected with EPV vectors containing late poxvirus promoters are supplied with certain additional factors *in trans* (*e.g.*, by co-infection with vaccinia virus), which at least partially rescue late gene expression through activation of late promoters.

5. However, as taught within the patent application, early poxvirus promoters and (under certain circumstances) late poxvirus promoters are not the only promoters that can be used in an entomopoxvirus vector to achieve expression of a foreign gene within a vertebrate cell. Entomopoxvirus vectors containing genes under the control of non-poxvirus promoters can also be utilized, such as vertebrate host cell promoters and promoters from other viruses. This is documented at page 75, lines 11-24, and page 81, lines 15-24, of the patent application. Specifically, non-poxvirus promoters that are recognized by the vertebrate host cell's nuclear RNA polymerase, such as the cytomegalovirus (CMV) and herpes TK gene promoters, can be used following entomopoxvirus infection, entry of the viral DNA into the nucleus, and selection for stable transformation of the vertebrate host cell. Once transformed, those foreign genes that are under the control of the non-poxvirus promoters within the nuclear environment become activated, leading to expression of the foreign gene. Preferred promoters are those constitutive or regulatable promoters, such as the CMV or Herpes TK gene promoters, capable of promoting sufficient expression of the foreign DNA contained within the viral vector in a vertebrate cell.

6. The patent Examiner states that Example 11 of the patent application only examines expression of β-galactosidase expression at day two, following injection, "and does not correlate the level or duration of gene expression with any therapeutic effect." However, it would be expected that expression of the

foreign gene, β-galactosidase in this case, would be sustained for a period beyond the two days at which time the tissue was excised.

7. The patent Examiner states the skilled artisan would not predict that the entomopox virus vectors of the invention could be used to express therapeutic levels of protein in lymphoid cells, which are associated with certain disorders, such as Burkitt's lymphoma. Although it has been reported in the literature that there is a greater level of expression in some cells (*e.g.*, fibroblasts) than in other cells, such as lymphoid cells, one would expect that greater levels of expression could be achieved, for example, through the use of tissue-specific promoters and/or *in vitro* selection steps.

8. In summary, given the teachings of the patent application, one would expect that the entomopoxvirus vectors of the invention can be used to deliver and express foreign genes within a vertebrate cell, using a variety of promoters.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Signed: Robert W. Moyer

Richard W. Moyer, Ph.D.

Date: January 11, 2002